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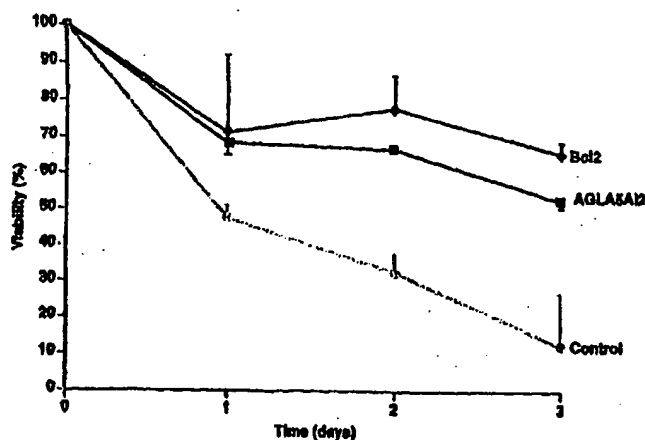


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(54) Title: A6LA5A12 POLYPEPTIDES AND POLYNUCLEOTIDES AND METHODS OF USE THEREFOR

**A6LA5A12 Acts as a Survival Factor for
Serum Starved Neurons**



(57) Abstract

Compositions and methods for enhancing cell survival are provided. Such compositions comprise A6LA5A12 polypeptides, and variants thereof, that inhibit stress-induced cell death. The polypeptides may be used, for example, in the prevention and treatment of a variety of conditions associated with oxidative damage and cell death.

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DescriptionA6LA5A12 POLYPEPTIDES AND POLYNUCLEOTIDES
AND METHODS OF USE THEREFOR

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Technical Field

The present invention relates generally to compositions and methods for enhancing cell survival. The invention is more particularly related to A6LA5A12 polypeptides and variants thereof that inhibit stress-induced cell death. Such polypeptides may be used, for example, in the prevention and treatment of a variety of conditions associated with oxidative stress and cell death.

Background of the Invention

Eukaryotic cells are exposed to numerous stresses that can inhibit normal cellular functions and lead to cell death. Such stresses include the lack of oxygen, excessive heat, viral infection, cytokines, drugs and highly reactive oxygen-derived free radicals. In response to such stresses, cells typically activate a variety of defense mechanisms. For example, the synthesis of a class of proteins known as heat shock proteins is induced in response to heat, and antioxidant molecules and enzymes are cellular defenses against oxygen free radicals. In healthy cells, such defenses effectively minimize stress-induced damage.

Cellular defenses against stresses can be weakened by a variety of diseases and injuries. In such cases, treatment with one or more compounds that promote cell survival may be beneficial. For example, sick or injured cells generally make increased amounts of superoxide anion, which is highly reactive with cellular components. This can lead to damage to, for example, membrane lipids, connective tissue and nucleic acids (for reviews, see Michels et al., *Free Radical Biol. and Med.* 17:235-248, 1994; Turrens, *Xenobiotica* 21:1033-1040, 1991). High levels of superoxide anions may contribute to diseases of the lung, central nervous system and skeletal muscle, as well as vascular disorders, inflammatory diseases and the aging process. The anti-oxidant superoxide dismutases (SODs) function *in vivo* to convert superoxide anions into H_2O_2 and O_2 . SODs and compounds that enhance the activity of these enzymes have been used to treat a variety of conditions, including severe head injury (see J.P. Mauzelzaar in *Free Radicals in Diagnostic Medicine*, D. Armstrong, ed., Plenum Press, NY,

1994); inflammatory diseases such as arthritis (*see* Jadot et al., *Clin. Pharm.* 28:17-25, 1995); bronchial pulmonary dysplasia (*see* U. S. Patent No. 5,540,911); muscle wasting disorders, neurological diseases and autoimmune diseases (*see* U.S. Patent No. 5,550,262); burns and radiation damage (*see* WO 96/14083); and cardiovascular disease, liver cirrhosis, diabetes, cataracts and aging (*see* WO 96/00790).

While such treatments may be beneficial, a variety of metabolic problems may result from excessive superoxide dismutase activity. It has been found, for example, that superoxide dismutases can lose their ability to protect ischemically injured isolated hearts when used at high doses (*see* McCord, *Clin. Biochem.* 26:351-357, 1993). Furthermore, a delicate balance between superoxide radicals and superoxide dismutases appears to be required for normal cell and organ function, and genetic overexpression of SOD can result in undesirable side-effects.

Accordingly, there is a need in the art for further compounds capable of inhibiting stress-induced cell damage and death. The present invention fulfills this need and further provides other related advantages.

Summary of the Invention

Briefly stated, the present invention provides A6LA5A12 polypeptides and methods employing such polypeptides. In one aspect, an isolated polynucleotide is provided. In one embodiment, the polynucleotide comprises a sequence selected from the group consisting of: (a) a sequence recited in SEQ ID NO:1; (b) a sequence recited in SEQ ID NO:3; (c) nucleotide sequences that hybridize to a polynucleotide complementary to SEQ ID NO:1 or SEQ ID NO:3 under moderately stringent conditions, wherein the nucleotide sequence encodes a polypeptide having an ability to enhance cell survival; and (d) nucleotide sequences that encode a polypeptide encoded by any of the foregoing sequences. In another embodiment, the polynucleotide encodes a polypeptide recited in SEQ ID NO:2 or SEQ ID NO:4, or a variant of such a polypeptide that is capable of enhancing cell survival.

In related aspects, the present invention provides a recombinant expression vector comprising a polynucleotide as described above and a host cell transformed or transfected with such an expression vector.

In another aspect, a method for preparing an A6LA5A12 polypeptide is provided, the method comprising culturing a host cell as described above under conditions promoting expression of the polynucleotide sequence and recovering an A6LA5A12 polypeptide.

5 The present invention also provides polypeptides comprising an amino acid sequence encoded by a polynucleotide as described above, as well as polypeptides comprising an amino acid sequence recited in SEQ ID NO:2 or SEQ ID NO:4, or a variant of such a sequence, wherein the polypeptide is capable of enhancing cell survival.

 In further aspects, methods for enhancing survival of a cell are provided. The
10 methods comprise increasing A6LA5A12 activity within a cell, and thereby enhancing survival of the cell. In one such aspect, A6LA5A12 activity is increased by inducing expression of an endogenous A6LA5A12 gene. In another aspect, the method comprises: (a) transfecting the cell with a polynucleotide as described above; and (b) promoting expression of the polynucleotide within the cell, thereby enhancing survival of the cell.

15 In another aspect, the present invention provides a pharmaceutical composition comprising a polypeptide as described above in combination with a pharmaceutically acceptable carrier.

 The present invention also provides methods for treating a patient afflicted with a condition associated with oxidative damage, comprising administering to a patient a
20 pharmaceutical composition as described above and thereby alleviating one or more symptoms of a condition associated with oxidative damage.

 In a related aspect, methods for treating a patient afflicted with a condition associated with cell death induced by stress are provided, comprising administering to a patient a pharmaceutical composition as described above, and thereby alleviating one or more
25 symptoms of a condition associated with cell death induced by stress.

 In further aspects, the present invention provides methods for identifying an agent that modulates A6LA5A12 activity, comprising: (a) contacting a candidate modulator with a polypeptide as describe above, wherein the step of contacting is carried out under conditions and for a time sufficient to allow the candidate modulator to interact with the
30 polypeptide; and (b) subsequently identifying a modulator that binds to said polypeptide, and therefrom identifying an agent that modulates A6LA5A12 activity.

In a related aspect, methods for modulating A6LA5A12 activity in a cell are provided, comprising contacting a cell expressing a polypeptide as described above with an effective amount of an agent that modulates A6LA5A12 activity.

The present invention also provides antibodies that bind to a polypeptide as described above.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

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Brief Description of the Drawings

Figure 1 presents the sequence of a human polynucleotide encoding a representative A6LA5A12 polypeptide.

Figure 2 presents the sequence of human endogenous A6LA5A12, a representative A6LA5A12 polypeptide.

Figure 3 presents the sequence of a rat polynucleotide encoding a representative A6LA5A12 polypeptide.

Figure 4 presents the sequence of rat endogenous A6LA5A12, a representative A6LA5A12 polypeptide.

Figure 5 is a graph showing the effect of a representative A6LA5A12 polypeptide (squares) on cell viability in serum starved neurons, compared to the positive control bcl12 (solid circles) and control vector (hollow circles).

Figure 6 is an autoradiogram showing the expression of A6LA5A12 mRNA in ischemia.

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Detailed Description of the Invention

As noted above, the present invention is generally directed to compositions and methods for enhancing cell survival by inhibiting stress-induced cell damage and death. The invention is more particularly related to a human A6LA5A12 polypeptide, and variants thereof. Such polypeptides may be used, for example, to enhance cell survival *in vitro* or *in vivo* and to prevent and/or treat conditions associated with cell death. Such conditions include

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diseases and injuries associated with oxidative stress, such as those that result from the deleterious effects of superoxide anions.

As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length endogenous (*i.e.*, native) A6LA5A12 proteins. An A6LA5A12 polypeptide may consist entirely of a native sequence, or may contain additional heterologous sequences. A variant of an A6LA5A12 polypeptide is a polypeptide that differs in sequence from an A6LA5A12 polypeptide only in substitutions, deletions and/or other modifications, such that the variant retains the ability to enhance cell survival (*i.e.*, administration of the polypeptide results in a statistically significant increase in cell viability, as determined using any method known to those of ordinary skill in the art, such as staining with trypan blue to measure live and dead cells).

Amino acid substitutions are preferably made at no more than 50% of the amino acid residues in the native polypeptide. Such substitutions are preferably conservative. A conservative substitution is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophobic nature of the polypeptide to be substantially unchanged. In general, the following amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. Substitutions, deletions and/or amino acid additions may be made at any location(s) in the polypeptide, provided that the modification does not diminish the ability of the variant to enhance cell survival. Thus, a variant may comprise only a portion of a native A6LA5A12 sequence. In addition, or alternatively, variants may contain additional amino acid sequences (such as, for example, linkers, tags and/or ligands), preferably at the amino and/or carboxy termini. Such sequences may be used, for example, to facilitate purification, detection or cellular uptake of the polypeptide.

In general, A6LA5A12 polypeptides, and polynucleotides encoding such polypeptides, may be prepared using any of a variety of techniques that are well known in the art. For example, a DNA sequence encoding native A6LA5A12 may be prepared by amplification from a suitable cDNA or genomic library using, for example, polymerase chain reaction (PCR) or hybridization techniques. Libraries may generally be prepared and screened using methods well known to those of ordinary skill in the art, such as those described in

Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989. It has been found, within the context of the present invention, that expression of this gene is induced in glial cells and neurons that are exposed to a stress or an activating agent (e.g., cytokines, heat, lack of oxygen, as well as beta amyloid and variations of aggregated beta amyloid, such as endotoxin). In rat astrocytes, for example, expression of the A6LA5A12 gene is induced by beta amyloid. Accordingly, cDNA libraries prepared from such stimulated cells are suitable for amplification of A6LA5A12. Other libraries that may be employed will be apparent to those of ordinary skill in the art.

Primers for use in amplification may be readily designed based on the sequence of a native human A6LA5A12 cDNA provided in Figure 1 and SEQ ID NO:1 and native rat A6LA5A12 cDNA, provided in Figure 3 and SEQ ID NO:3. The human and rat cDNAs are 66% identical at the nucleotide level. The sequence of the encoded native human and rat polypeptides are provided in Figure 2 and SEQ ID NO:2, and Figure 4 and SEQ ID NO:4, respectively. These polypeptides are 90% identical.

Polynucleotides encompassed by the present invention include DNA and RNA molecules that comprise a sequence recited in SEQ ID NO:1 or SEQ ID NO:3. Also encompassed are other polynucleotides that encode an A6LA5A12 amino acid sequences provided in SEQ ID NO:2 or SEQ ID NO:4, as well as polynucleotides that encode variants of the A6LA5A12 sequence that retain the ability to enhance cell survival. Polynucleotides that are substantially homologous to a sequence provided in SEQ ID NO:1 or SEQ ID NO:3 are also within the scope of the present invention. "Substantial homology," as used herein refers to polynucleotides that are capable of hybridizing under moderately stringent conditions to a polynucleotide complementary to the sequence provided in SEQ ID NO:1 or SEQ ID NO:3, provided that the encoded A6LA5A12 variant enhances cell survival. Suitable moderately stringent conditions include prewashing in a solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50-65°C, 5X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS. Nucleotide sequences that, because of code degeneracy, encode a polypeptide encoded by any of the above sequences are also encompassed by the present invention.

Polypeptides of the present invention may be prepared by expression of recombinant DNA encoding the polypeptide in cultured host cells. Preferably, the host cells

are bacteria, yeast, insect or mammalian cells. The recombinant DNA may be cloned into any expression vector suitable for use within the host cell and transfected into the host cell using techniques well known to those of ordinary skill in the art. An expression vector generally contains a promoter sequence that is active in the host cell. A tissue specific promoter may
5 also be used, as long as it is activated in the target cell. Preferred promoters express the polypeptide at high levels.

Optionally, the construct may contain an enhancer, a transcription terminator, a poly(A) signal sequence, a bacterial or mammalian origin of replication and/or a selectable marker, all of which are well known in the art. Enhancer sequences may be included as part of
10 the promoter region used or separately. Transcription terminators are sequences that stop RNA polymerase-mediated transcription. The poly(A) signal may be contained within the termination sequence or incorporated separately. A selectable marker includes any gene that confers a phenotype on the host cell that allows transformed cells to be identified. Such markers may confer a growth advantage under specified conditions. Suitable selectable
15 markers for bacteria are well known and include resistance genes for ampicillin, kanamycin and tetracycline. Suitable selectable markers for mammalian cells include hygromycin, neomycin, genes that complement a deficiency in the host (*e.g.* thymidine kinase and TK⁻cells) and others well known in the art.

DNA sequences expressed in this manner may encode native human
20 A6LA5A12, or may encode portions or other variants of native A6LA5A12. DNA molecules encoding variants of A6LA5A12 may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis, and sections of the DNA sequence may be removed to permit preparation of truncated polypeptides. As noted above, a variant within the scope of the present invention is capable of enhancing cell
25 survival. The ability of a variant to enhance cell survival may generally be measured by any of a variety of techniques well known to those of ordinary skill in the art for evaluating cell viability. For example, staining with trypan blue may be used to measure live and dead cells. Other assays known to those of ordinary skill in the art for evaluating a compound's ability to protect against oxidative damage may also be employed. In one such assay, animals or cells
30 are exposed to a free radical generating system with and without administration of a test compound and evaluating the percent protection using, for example, a dose-response

protection curve. Other suitable assays will be apparent to those of ordinary skill in the art, and are described, for example, in Michels et al., *Free Radical Biol. and Med.* 17:235-248, 1994 and references cited therein.

5 Variants of A6LA5A12 include constitutively active proteins. In general, activation of A6LA5A12 *in vivo* requires stimulation by stress-inducing or activating agents. Constitutively active variants display the ability to enhance cell survival in the absence of such stimulation. Such variants may be readily identified using the representative assay described herein. Genes encoding proteins modified so as to be constitutively active may generally be used in the treatment of a variety of conditions, as discussed in more detail below.

10 A6LA5A12 polypeptides may be expressed in transfected cells by culturing the cell under conditions promoting expression of the transfected polynucleotide. Appropriate conditions will depend on the specific host cell and expression vector employed, and will be readily apparent to those of ordinary skill in the art. For commercially available expression vectors, the polypeptide may generally be expressed according to the manufacturer's
15 instructions. Expressed polypeptides of this invention are generally isolated in substantially pure form. Preferably, the polypeptides are isolated to a purity of at least 80% by weight, more preferably to a purity of at least 95% by weight, and most preferably to a purity of at least 99% by weight. In general, such purification may be achieved using, for example, the standard techniques of ammonium sulfate fractionation, SDS-PAGE electrophoresis, and/or
20 affinity chromatography.

As noted above, expression of native human A6LA5A12 is induced in glial cells and neurons that are exposed to a stress or an activating agent. In addition, it has been found within the context of the present invention that A6LA5A12 polypeptides serve as a protective factor for neurons that are subsequently placed under stress. Briefly, a neuronal cell
25 line that is susceptible to serum starvation shows a significant increase in viability following serum starvation when transfected with an expression construct containing an A6LA5A12 polypeptide. Accordingly, A6LA5A12 polypeptides may be employed, alone or in combination with other therapies, to treat or prevent conditions in which the enhancement of cell survival is beneficial. In particular, A6LA5A12 polypeptides may be used for the
30 treatment of conditions associated with oxidative stress, such as conditions resulting from defects in superoxide dismutase (SOD) activity or from a need for enhanced SOD activity.

Defects in SOD activity have been implicated in the pathogenesis of some disorders, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, stroke, trauma, amyotrophic lateral sclerosis and ischemia. Other conditions associated with defects in SOD activity may generally be identified by, for example, comparing the level of SOD expression within a diseased cell or tissue to the level observed in a healthy cell or tissue. Such evaluations may be readily performed using standard techniques, and as described in Borrello et al., *Molec. Aspects. Med.* 14:253-258, 1993, and references cited therein.

Conditions for which treatment with an A6LA5A12 polypeptide may be beneficial (for therapy and/or prevention) are those conditions that are associated with cell death induced by stress. In particular, such conditions that are associated with oxidative damage may be treated. Such conditions include CNS disorders such as Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, Hallervorden-Spatz disease, diffuse lewy body disease, torsion dystonia, familial tremor, Tourette's syndrome and stroke; ischemic and traumatic brain injuries; memory disorders; endometriosis; gout; inflammatory diseases such as arthritis, synovial inflammation, inflammatory bowel disease, synovial inflammation and inflammation and toxicity resulting from ionizing radiation, ischemia and tumors; reperfusion injury; rheumatoid arthritis; bronchial pulmonary dysplasia; respiratory diseases such as bronchitis, acute respiratory distress syndrome, infantile respiratory distress, asthma and emphysema; fibrosis; muscle wasting disorders; weight loss; autoimmune diseases; skin disorders such as burns, radiation damage and acne; cancer; hair loss; impotence; neonate hemorrhages; cardiovascular disease; arrhythmia; hypertension; ulcers; liver cirrhosis; organ transplant rejection; diabetes; thrombosis; fatty embolism; sickle cell anemia; perfusion injury; leprosy; malnutrition; oedema and paraquat intoxication; cataracts; retinopathy; viral infections such as HIV and herpes; nonvascular gastrointestinal disorders and aging.

Enhancement of cell survival, either *in vitro* or *in vivo*, may generally be achieved by (1) increasing the activity of exogenous A6LA5A12 within the cell, (2) transfection of the cell with a polynucleotide encoding A6LA5A12 or a variant thereof, and promoting expression of the polynucleotide within the cell or (3) treatment of the cell with an A6LA5A12 polypeptide, or variant thereof. In some instances, however, the overexpression of A6LA5A12 may be deleterious. In such cases, modulating agents that inhibit A6LA5A12

function may be administered. Each of these methods may be performed using mammalian cells in culture or within a mammal, such as a human. The above methods are described in greater detail below.

The activity of endogenous A6LA5A12 within a cell may be increased by
5 inducing expression of the A6LA5A12 gene and/or administering a modulating agent that enhances A6LA5A12 activity. To induce expression of an endogenous A6LA5A12 gene, any of a variety of stresses or activating agents may be employed. Preferably, an A6LA5A12 gene is induced by an activating agent such as a cytokine.

A modulating agent that enhances A6LA5A12 activity may be any compound
10 that increases the rate of synthesis of A6LA5A12, stabilizes A6LA5A12 and/or increases the activity of the endogenous A6LA5A12. To identify such an agent, any of a variety of screens may be performed. Candidate modulating agents may be isolated using well known techniques from a variety of sources, such as plants, fungi or libraries of chemicals, small molecules or random peptides. Preferably, a modulating agent has a minimum of side effects and is non-
15 toxic. For some applications, agents that can penetrate cells are preferred.

An initial screen to assess the ability of candidate agents to bind to A6LA5A12 may be employed, although such binding is not essential for a modulating agent. For identifying agents that bind to an A6LA5A12 polypeptide, any of a variety of binding assays may be employed, such as standard affinity techniques and yeast two-hybrid screens. In
20 general, the amount of candidate modulator added in such screens ranges from about 1 pM to 1 μ M.

Screens for modulating agents that increase the rate of A6LA5A12 synthesis or stabilize A6LA5A12 may be readily performed using well known techniques that detect the level of A6LA5A12 protein or mRNA. Suitable assays include RNA protection assays, *in situ*
25 hybridization, ELISAs, Northern blots and Western blots. Such assays may generally be performed using standard methods (*see* Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). For example, to detect mRNA encoding A6LA5A12, a nucleic acid probe complementary to all or a portion of the A6LA5A12 gene sequence may be employed in a Northern blot analysis of mRNA
30 prepared from suitable cells. To detect A6LA5A12 protein, a reagent that binds to the protein (typically an antibody) may be employed within an ELISA or Western assay. Following

binding, a reporter group suitable for direct or indirect detection of the reagent is employed (*i.e.*, the reporter group may be covalently bound to the reagent or may be bound to a second molecule, such as Protein A, Protein G, immunoglobulin or lectin, which is itself capable of binding to the reagent). Suitable reporter groups include, but are not limited to, enzymes
5 (e.g., horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. Such reporter groups may be used to directly or indirectly detect binding of the reagent to a sample component using standard methods known to those of ordinary skill in the art.

To use such assays for identifying a modulating agent, the level of A6LA5A12
10 protein or mRNA is evaluated in cells treated with one or more candidate modulating agents. An increase or decrease in A6LA5A12 levels may be measured by evaluating A6LA5A12 mRNA and/or protein in the presence and absence of candidate modulating agent. In general, the amount of candidate modulator added in such screens ranges from about 1 pM to 1 μ M. A candidate that results in a statistically significant change in the level of A6LA5A12 mRNA
15 and/or protein is a modulating agent.

Modulating agents that increase A6LA5A12 levels generally enhance A6LA5A12 activity. To further evaluate the effect on A6LA5A12 activity, cell survival may be evaluated as described above in the presence and absence of modulating agent. Such modulating agents may be administered by addition to a cell culture or by the methods
20 described below for *in vivo* administration.

To enhance cell survival, either *in vitro* or *in vivo*, using a polynucleotide encoding an A6LA5A12 polypeptide or a variant thereof, cells may be transfected using any of a variety of techniques known in the art. Such transfection may result in stable transformants or may be transient. One suitable transfection technique is electroporation, which may be
25 performed on a variety of cell types, including mammalian cells, yeast cells and bacteria, using commercially available equipment. Optimal conditions for electroporation (including voltage, resistance and pulse length) are experimentally determined for the particular host cell type. General guidelines for optimizing electroporation may be obtained from manufacturers or found in protocol manuals, such as *Current Protocols in Molecular Biology*, Ausubel et al.
30 (ed.), Wiley Interscience, 1987.

Alternatively, the polynucleotide may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid, bacterial and viral expression systems, and colloidal dispersion systems such as liposomes. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal, as described above). The DNA may also be "naked," as described, for example, in Ulmer et al., *Science* 259:1745-1749, 1993.

Various viral vectors that can be used to introduce a nucleic acid sequence into the targeted patient's cells include, but are not limited to, vaccinia or other pox virus, herpes virus, retrovirus, or adenovirus. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus including, but not limited to, Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a gene that encodes the ligand for a receptor on a specific target cell (to render the vector target specific).

Viral vectors are typically non-pathogenic (defective), replication competent viruses, which require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids that encode all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR, but that are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsulation. Such helper cell lines include (but are not limited to) Ψ2, PA317 and PA12. A retroviral vector introduced into such cells can be packaged and vector virion produced. The vector virions produced by this method can then be used to infect a tissue cell line, such as NIH 3T3 cells, to produce large quantities of chimeric retroviral virions.

Another targeted delivery system for A6LA5A12 polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). RNA, DNA and

intact virions can be encapsulated within the aqueous interior and delivered to cells in a biologically active form (*see* Fraley, et al., *Trends Biochem. Sci.* 6:77, 1981). The preparation and use of liposomes is well known to those of ordinary skill in the art.

Following transfection, cells are maintained in conditions that promote
5 expression of the polynucleotide within the cell. As discussed above, appropriate conditions depend upon the expression system and cell type, and will be apparent to those skilled in the art.

To enhance cell survival by direct administration of an A6LA5A12 polypeptide, or a variant thereof, the polypeptide may be administered such that it is capable of entry into
10 the target cell. For example, a polypeptide may be designed to bind to a surface receptor present on the target cell. Those of ordinary skill in the art will be readily able to identify sequences that bind to a receptor present on any given cell type. Such sequences may be incorporated into a polypeptide, as described above, or associated with the polypeptide using techniques familiar to those of ordinary skill in the art to permit uptake of the polypeptide into
15 the target cell.

Alternatively, to treat a patient afflicted with certain conditions associated with oxidative damage (such as inflammatory ailments), it may be beneficial to deliver an A6LA5A12 polypeptide to the intracellular space. Such targeting may be achieved using well known techniques, such as through the use of polyethylene glycol or liposomes, as described
20 in Turrens, *Xenobiotica* 21:1033-1040, 1991.

As noted above, modulating agents that inhibit A6LA5A12 function may be administered in cases where overexpression of A6LA5A12 is undesirable. Such inhibitory modulating agents generally bind to A6LA5A12 and inhibit its activity. Accordingly, an initial screen for such agents may be performed using a binding assay, as described above. Candidate
25 agents that bind to A6LA5A12 may then be evaluated for their effect on the enhancement of cell survival by an A6LA5A12 polypeptide, using the representative assays described herein. Modulating agents that inhibit A6LA5A12 function are those that result in a decrease in A6LA5A12-induced enhancement of cell survival in such assays.

For *in vivo* use, A6LA5A12 polypeptides, polynucleotides and/or modulating
30 agents are generally incorporated into a pharmaceutical composition prior to administration. A pharmaceutical composition comprises one or more such compounds in combination with a

physiologically acceptable carrier. Pharmaceutical carriers include any such carriers known to those skilled in the art to be suitable for the particular mode of administration, and may be liquid, semi-liquid or solid form.

Administration may be achieved by a variety of different routes, including oral,
5 parenteral, nasal, intravenous, intradermal, subcutaneous or topical. Preferred modes of administration depend upon the condition to be treated or prevented. For example, dermatological and ophthalmologic conditions will typically be treated locally. Compositions applied topically, such as to the skin and/or mucous membranes, may be in the form of a gel or cream, and solutions intended for ophthalmic use may be formulated as isotonic solutions.
10 Other conditions may be treated by systemic, intradermal or intramuscular modes of administration.

To prepare a pharmaceutical composition, an effective amount of one or more polypeptides, polynucleotides and/or modulating agents is mixed with a suitable pharmaceutical carrier. An amount that, upon administration, ameliorates the symptoms or
15 treats or delays or prevents progression of the condition is considered effective. Administration may be effected by incubation of cells *ex vivo* or *in vivo*, such as by topical treatment, delivery by specific carrier or by vascular supply. The precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and
20 extrapolating therefrom. Dosages may also vary with the severity of the condition to be alleviated. The composition may be administered one time, or may be divided into a number of smaller doses to be administered at intervals of time. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need.

Solutions or suspensions used for parenteral, intradermal, subcutaneous or
25 topical application can include, for example, a sterile diluent (such as water), saline solution, fixed oil, polyethylene glycol, glycerine, propylene glycol or other synthetic solvent; antimicrobial agents (such as benzyl alcohol and methyl parabens); antioxidants (such as ascorbic acid and sodium bisulfite) and chelating agents (such as ethylenediaminetetraacetic acid (EDTA)); buffers (such as acetates, citrates and phosphates). If administered
30 intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene

glycol, polypropylene glycol and mixtures thereof. In addition, other pharmaceutically active ingredients and/or suitable excipients such as salts, buffers and stabilizers may, but need not, be present within the composition.

5 A polypeptide, polynucleotide or modulating agent may be prepared with carriers that protect it against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others known to those of ordinary skill in the art.

10 A pharmaceutical composition is generally formulated and administered to exert a therapeutically useful effect while minimizing undesirable side effects. The number and degree of acceptable side effects depend upon the condition for which the composition is administered. For example, certain toxic and undesirable side effects that are tolerated when treating life-threatening illnesses, such as tumors, would not be tolerated when treating disorders of lesser consequence. The concentration of active component in the composition
15 will depend on absorption, inactivation and excretion rates thereof, the dosage schedule and the amount administered, as well as other factors that may be readily determined by those of skill in the art.

In another aspect of the present invention, an A6LA5A12 polypeptide or
20 variant thereof may be used to bind heavy metals (preferably copper, zinc and/or cobalt) extracellularly. In this regard, A6LA5A12 polypeptides are more easily expressed and used than currently available heavy metal binding proteins because of their small size. Such polypeptides may be used within any methods known in the art for which heavy metal binding is desirable, including assays to detect heavy metals in solution. A6LA5A12 polypeptides may
25 also be used for removal of heavy metals *in vitro* or *in vivo*.

A6LA5A12 may also be used to enhance the effect of one or more anticancer drugs. In particular, A6LA5A12 may support the survival of normal cells and/or have a deleterious effect on cancer cells when used in combination with other anticancer agents.

Also provided within the present invention are antibodies that bind to human
30 A6LA5A12. Such antibodies may be polyclonal or monoclonal, and are specific for human A6LA5A12 and/or one or more variants thereof. Preferred antibodies are those antibodies

that function as modulating agents to inhibit or block A6LA5A12 activity *in vivo*. Antibodies may also be employed within assays for detecting the level of A6LA5A12 within a sample.

Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art (*see, e.g., Harlow and Lane, Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988). In one such technique, an immunogen comprising the polypeptide is initially injected into a suitable animal (*e.g., mice, rats, rabbits, sheep and goats*), preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e., reactivity with the polypeptide of interest*). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction.

Antibodies and other agents having a desired effect on A6LA5A12 activity, as described above, may be administered to a patient (either prophylactically or for treatment of

an existing disease) to modulate the activity of A6LA5A12 *in vivo*. In general, for administration to a patient, an antibody or other agent is formulated as a pharmaceutical composition as described above. A pharmaceutical composition may also, or alternatively, contain DNA encoding an antibody or other agent as described above, such that the active agent is generated *in situ*. In such pharmaceutical compositions, the DNA may be introduced using any of a variety of delivery systems known to those of ordinary skill in the art, such as those described above. For administration of such agents, routes, frequency and doses will vary from patient to patient. In general, however, the pharmaceutical compositions may be administered as described above. A suitable dose of such an agent is an amount sufficient to show benefit in the patient based on the criteria noted above.

In a related aspect of the present invention, kits for detecting A6LA5A12 are provided. Such kits may be designed for detecting the level of A6LA5A12 or nucleic acid encoding A6LA5A12 within a sample. In general, the kits of the present invention comprise one or more containers enclosing elements, such as reagents or buffers, to be used in the assay. A kit for detecting the level of A6LA5A12, or nucleic acid encoding A6LA5A12, typically contains a reagent that binds to the A6LA5A12 protein, DNA or RNA. To detect nucleic acid encoding A6LA5A12, the reagent may be a nucleic acid probe or a PCR primer. To detect A6LA5A12 protein, the reagent is typically an antibody. The kit also contains a reporter group suitable for direct or indirect detection of the reagent as described above.

20

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1Preparation of A6LA5A12 cDNA

5 This Example illustrates the preparation of a cDNA encoding native human and rat A6LA5A12 proteins.

A. Isolation of Rat Astroglial Cells

 Newborn rats were sacrificed and the brain aseptically removed from the skull.

10 The neocortex was isolated and the meninges carefully removed. The tissue was then mechanically dissociated and seeded in culture flasks in DMEM supplemented with glutamine and 10% fetal calf serum at a density of about 2×10^5 cells/cm². The cells were incubated in a 5% CO₂ incubator at 37°C. The medium was changed every 3 to 4 days until the cells reached confluence (usually about 8 to 14 days). After the cells were confluent, the flasks were placed

15 on a rotary shaker at 240 rpm over night. The supernatant which contains the microglia was aspirated the following day. The cells were allowed to recover in regular DMEM plus serum for 1 day. They were then treated with 10mM Leucine Methyl Ester (LME) in serum free DMEM and shaken at 200 rpm for at least 6 hours. The medium was aspirated and the cells were allowed to recover overnight in DMEM plus serum. The exact same procedure with

20 LME was repeated the next day. This procedure yielded a nearly pure (>98%) population of astrocytes.

B. Treatment of Astrocytes with Aggregated Beta Amyloid and Isolation of RNA

 Astrocytes were either treated or untreated with 8.7mM aggregated beta

25 amyloid (1-42) for 24 hours. RNA was then extracted from the cells using Trizol (Gibco, Gathersburg, MD) as recommended by the manufacturer. PolyA⁺ mRNA was isolated using FastTrack (Invitrogen, San Diego, CA).

C. Subtractive Hybridization

30 *cDNA Digestion.* 200 ng of driver cDNA library was digested with BstXI, NotI, and ScaI. 10 ng of tracer cDNA library was digested with BstXI and NotI. To the

driver cDNA reaction 8 ml of 25mM dNTP's and 20 ml of Klenow (100 U/ μ l) were added, the mixture was incubated at 37°C for 30 minutes, and inactivated at 70°C for 10 minutes. Both the driver and tracer cDNA were phenol:chloroform:isoamyl alcohol extracted. The aqueous phase was precipitated with 3 M ammonium acetate and 2.5 volumes of 100% ethanol, and microfuged at 15000 rpm for 20 minutes. The driver cDNA digest was resuspended in 200 μ l water. The tracer cDNA digest was resuspended in 8 μ l of TE, pH 8.0.

Photobiotinylation. To 200 μ l (1 μ g/ μ l) of the driver cDNA, 200 μ l of photoprobe long arm biotin (Vector Labs, Burlingame, CA) resuspended in 500 μ l of water was added to the cDNA in the dark and the cDNA was exposed to a 270 watt lamp, from a 10 cm distance for 20 minutes on ice. The top of the tube was left open. An additional 100 μ l of photoprobe biotin was added to cDNA and the solution was exposed under the lamp for an additional 20 minutes. 50 μ l of 1M Tris, pH 9.5, and 500 μ l of water-saturated n-butanol was added and the mixture was vortexed. The upper brown n-butanol phase was discarded, and the aqueous phase was extracted with n-butanol a total of 5 times. The aqueous phase was heated at 45°C for 10 minutes. 42 μ l of 3M ammonium acetate was added, and the mixture was divided into two tubes, precipitated with 700 μ l of ethanol in each tube, and microfuged for 20 minutes at 14,000 rpm. The brown biotinylated pellet was washed with 70% ethanol and resuspended in 30 μ l of TE, pH 7.8.

Hybridization. To set up the long hybridization 15 μ l (100 ng) of biotinylated driver cDNA, 4 μ l (5 ng) of tracer cDNA and 1 μ l of tRNA (10 ng) were placed in a 0.5 ml PCR tube and heated at 100°C for three minutes. 20 μ l of 2X hybridization buffer (1.5M NaCl, 10mM EDTA, 50mM HEPES, and 0.2 % SDS) pre-warmed to 68°C was added to the tube, and the mixture was overlaid with 1 drop of paraffin oil. Hybridization mixtures were boiled for five minutes, and placed at 68°C for twenty hours (overnight). The hybridization reaction was removed from 68°C, 260 μ l of HEPES (10mM HEPES, 1mM EDTA) was added and the reaction was heated at 55°C for five minutes. The aqueous bottom phase was transferred to a new 0.5 ml Eppendorf tube. 20 μ l of Streptavidin (Pierce cat. no. 21122) resuspended at 2 μ g/ μ l in 0.15M NaCl, 10mM HEPES, and 1mM EDTA was added, and the reaction was gently mixed by finger vortexing. The tube was set at room temperature for 20 minutes. The reaction was extracted with 300 μ l of phenol:chloroform and microfuged for

five minutes. The top aqueous layer was transferred to a new tube and 20 μ l of Streptavidin was added. Incubation with Streptavidin and extraction with phenol:chloroform were repeated a total of four times. After final phenol:chloroform extraction aqueous layer was transferred to a new tube and extracted with 300 μ l of chloroform. The aqueous layer was removed to a new Eppendorf tube, and 25 μ l of 3M ammonium acetate and 813 μ l of ethanol were added. cDNA was precipitated at 15000 rpm for 15 minutes, washed with 70% ethanol, and resuspended in 11.5 μ l of TE, pH 8.0. The 11.5 μ l of cDNA was transferred to a 0.5 ml GeneAmp tube, 7.5 μ l (50 ng) of biotinylated driver cDNA and 1 μ l tRNA (10 ng) were added, and the mixture was boiled for 3 minutes. 20 μ l of prewarmed 2X hybridization buffer was added and the reaction was overlaid with 2 drops of paraffin oil. Short hybridization was set at 68°C for two hours. 260 μ l of HEPES was added, and the reaction was heated at 55°C for 5 minutes. The aqueous bottom layer was transferred to a new Eppendorf tube. Streptavidin was added and the reaction was incubated for 20 minutes and phenol:chloroform extracted. The process was repeated a total of 4 times exactly as described after long hybridization. After the last phenol:chloroform extraction, aqueous layer was transferred to a new tube, extracted with 300 μ l of chloroform, precipitated with 25 μ l of 3M ammonium acetate and 813 μ l of ethanol, microfuged at 15000 rpm for 15 minutes and washed with 70% ethanol. The pellet was resuspended in 5 μ l of TE, pH 8.0.

Preparation of the vector. 10 μ g of PCRII plasmid (Invitrogen, San Diego, CA) was digested with BstXI at 45°C for 3 hours, then NotI was added and incubation was continued at 37°C overnight. The digest was run on 1% low melting agarose gel and the 3.9 kb fragment was extracted. The fragment was dephosphorylated with 1 μ l of calf intestine phosphatase with 5 μ l of 10X buffer in a 50 μ l reaction at 37°C for 15 minutes. 1mM EDTA was added, the mixture was heated at 70°C for 10 minutes, loaded on 1 % low melting gel and the 3.9 kb fragment was purified.

Ligation. 5 μ l of subtracted cDNA was ligated to 250 ng PCRII (BstXI/NotI/CIP treated) with 1 μ l of 10X ligation buffer and 1 μ l of T4 ligase (400 U/10 μ l) in a 10 μ l volume. Incubation was performed at 16°C overnight. The ligation was precipitated with 1 μ l (10 ng) tRNA, 3M ammonium acetate, and 100% ethanol. The pellet was washed with 70% ethanol and the cDNA was resuspended in 4 μ l of water. 2 μ l of

ligated cDNA was transformed with 50 µl of Electromax DH10B cells (Gibco, Gaithersburg, MD) by electroporation (as specified by manufacturer). Transformed cells were cultured at 37°C for one hour in 1 ml of LB media. The transformation was diluted 1:10 and 1, 10, and 100 µl were plated on LB agar kanamycin plates. Remaining transformation was used to
5 inoculate a 1 liter culture for plasmid preparation.

The rat A6LA5A12 cDNA had the sequence shown in Figure 3 and SEQ ID NO:3. The encoded polypeptide is shown in Figure 4 and SEQ ID NO:4. A human A6LA5A12 cDNA was isolated by screening a human cDNA library with a the rat cDNA as a probe, using standard techniques. The sequence of the human cDNA is provided in SEQ ID
10 NO:1 and Figure 1, and the encoded polypeptide is provided in SEQ ID NO:2 and Figure 2.

Example 2

Protection of Neurons During Stress by Human A6LA5A12

This Example illustrates the use of a representative A6LA5A12 polypeptide to
15 protect neurons from stress-induced cell death.

A neuronal cell line (GT1-7) was cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum in 5% CO₂. Cells were grown to confluence, harvested and used for transfection. A retroviral expression construct (pBabe Puro) containing the puromycin gene for selection was used and the A6LA5A12 gene was inserted
20 into a convenient Bam - BglII site in the polylinker. Cells were infected with either the vector, a positive control construct in which the bcl2 gene was inserted or the construct containing the A6LA5A12 gene. For experiments, cells were harvested and plated on 100-mm diameter culture plates at 15-20% confluence and then allowed to grow to 50-70% confluence. To induce cell death serum supplemented medium was replaced with DMEM. The cells were
25 then assayed on each day for viability using trypan blue exclusion. Percentage viability was determined by comparing the average total cell number of a given treatment group with the average total cell number from the group treated for the identical time with control medium. For all conditions, viability assays were performed on triplicate dishes.

As shown in Figure 5, the A6LA5A12 polypeptide substantially increased cell
30 viability in serum starved neurons, indicating that this polypeptide is capable of enhancing cell survival.

Example 3

Induction of A6LA5A12 in Stroke Model

This Example illustrates the induction of A6LA5A12 in the middle cerebral
5 occlusion model for stroke.

A. Middle Cerebral Artery Occlusion Model

Rats were anesthetised initially with 4% halothane, and thereafter ventilated mechanically with a nitrous oxide - oxygen mixture (70%:30%) containing 0.5-1% halothane.

10 A tracheostomy was performed and polyethylene catheters inserted into both femoral arteries and one femoral vein to permit the continuous monitoring of blood pressure and the repeated sampling of arterial blood for determination of respiratory status and plasma glucose. Inspired halothane concentration was adjusted to 0.5-1% to produce adequate anaesthesia and to maintain mean arterial blood pressure (MABP) at 80 mmHg and above prior to drug/vehicle

15 administration. Respiratory volume was adjusted to maintain arterial carbon dioxide tension (PCO₂) between 34 and 42 mmHg. Adequate arterial oxygenation (PO₂ > 100mm Hg) was maintained throughout the experiment. Body temperature was monitored by a rectal thermometer, and the animals maintained normothermic (37±0.5°C) via a heating blanket controlled by the thermometer. Extreme caution was taken to avoid any procedure (e.g.,

20 direct heating of the head) which could artefactually alter the gradient between deep body temperature and brain temperature. All animals underwent a subtemporal craniectomy and exposure of the middle cerebral artery under an operating microscope at high magnification leaving the zygomatic arch intact (Tamura et al., *J. Cereb. Blood Flow Metab.* 1: 53-60, 1981). The exposed artery was occluded with microbipolar coagulation from its origin to its

25 junction with the inferior cerebral vein, and severed proximal to the lenticulostriate branches to assure completeness of the vascular occlusion.

B. In situ Hybridization Histochemistry

Dissected tissue was frozen in isopentane cooled to -42°C, and subsequently
30 stored at -80°C prior to sectioning on a cryostat. Slide mounted tissue sections were then

stored at -80°C . Sections were removed from storage and placed directly into 4% buffered paraformaldehyde at room temperature. After 60 minutes slides were rinsed in isotonic phosphate-buffered saline and treated with proteinase K (1 µg/ml) for 10 minutes at 37°C. Subsequently, sections underwent successive washes in water, 0.1 M triethanolamine (pH 8, plus 0.25% acetic anhydride) for 10 minutes, followed by treatment with 2 x SSC for 5 minutes. Sections were then dehydrated through graded alcohols and air dried. Postfixed sections were then hybridized with 1.0×10^6 dpm ^{35}S -UTP-labeled riboprobe in hybridization buffer containing 75% formamide, 10% dextran sulfate, 3 x SSC 50 mM sodium phosphate buffer (pH 7.4), 1 x Denhardt's solution, 0.1 mg/ml yeast tRNA and 10mM DDT. The diluted probe was applied to sections on a glass coverslip and hybridized overnight at 55°C in a humid environment. Posthybridization sections were washed in 2 x SSC for 5 minutes and then treated with RNase A (200ug/ml) for 60 minutes at 37°C. Subsequently, sections were washed in 2 x SSC for 5 minutes, 1 x SSC for 5 minutes, 0.1 x SSC for 60 minutes at 70°C, 0.5 x SSC at room temperature for 5 minutes and then dehydrated in graded alcohol and air dried. For signal detection, sections were placed on Kodak Bio Max x-ray film and exposed for the required length of time. Autoradiograms were analyzed using automated image analysis.

The results are presented in Figure 6. As shown in Figure 6, expression of A6LA5A12 was observed in the piriform cortex as detected by in situ hybridization. In contrast, the control portion exhibited no such expression.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Kelner, Gregory S.
Maki, Richard A.

(ii) TITLE OF INVENTION: A6LA5A12 POLYPEPTIDES AND POLYNUCLEOTIDES AND
METHODS OF USE THEREFOR

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:

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(D) STATE: Washington
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(F) ZIP: 98104

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE: 03-DEC-1996
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Maki, David J.
(B) REGISTRATION NUMBER: 31,392
(C) REFERENCE/DOCKET NUMBER: 690068.436

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (206) 622-4900

(B) TELEFAX: (206) 682-6031

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 425 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CACCGCGNCN ACACCGCCGC TGCCTCAGTC ATGCCGAAGC ACGAGTTCTC TGTGGACATG	60
ACCTGTGGAG GCTGTGCTGA AGCTGTCTCT CGGGTCCTCA ATAAGCTTGG AGGAGTTAAG	120
TATGACATTG ACCTGCCCAA CAAGAAGGTC TGCATTGAAT CTGAGCACAG CATGGACACT	180
CTGCTTGCAA CCCTGAAGAA AACAGGAAAG ACTGTTTCCT ACCTTGGCCT TGAGTAGCAG	240
GGGCCTGGTC CCCACAGCCC ACAGGATGGA CCAAAGGGGG CAGGATGCTG ATCCTCCCGC	300
TGGCTTCCAG ACAGACCTGG GACTTGGCAG TCATGCCGGG TNGATGGTGT TCCTGCGGAG	360
ACCCTCAAGT TTGTCCTATT CCTTTCCTAG CTTCCTGCA ATAAATCAA GCTGCTTTTG	420
TTGGA	425

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 68 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Pro Lys His Glu Phe Ser Val Asp Met Thr Cys Gly Gly Cys Ala
1 5 10 15

Glu Ala Val Ser Arg Val Leu Asn Lys Leu Gly Gly Val Lys Tyr Asp
 20 25 30

Ile Asp Leu Pro Asn Lys Lys Val Cys Ile Glu Ser Glu His Ser Met
 35 40 45

Asp Thr Leu Leu Ala Thr Leu Lys Lys Thr Gly Lys Thr Val Ser Tyr
 50 55 60

Leu Gly Leu Glu
65

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 444 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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CTGTGCCGCC TGTGCCGCCT GTGCCGCGTC AGTCATGCCG AAGCACGAGT TCTCTGTGGA      60
CATGACCTGT GGAGGCTGTG CGGAAGCCGT CTCCCGAGTC CTCAACAAGC TGGGAGGAGT      120
GGAGTTCAAC ATTGACTTGC CCAACAAGAA GGTCTGCATC GAGTCTGAGC ACAGCTCAGA      180
CATCCTGCTG GCAACTCTCA ACAAACAGG AAAAGCGGTC TCCTACCTTG GCCCAAGTA      240
GCCAGGACCT GGGCGAGTCC TTCCGGATAG AACTGAAGA GGCAGACTGT TGATCTGGTC      300
TCCCCCGCTG ATCTGGAACA CCAACTGCTC AGTCCAGCTC CGCCATGGAA TTCCAGCCCA      360
GACAGGCCTT CCCTGCTGGC TCCCTGCCAG CTCCTTGCA ATTAAAGTCA AGCTGCATTT      420
GTTAAAAAAA AAAAAAAAAA AAAA                                         444
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 67 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Pro Lys His Glu Phe Ser Val Asp Met Thr Cys Gly Gly Cys Ala
1           5           10          15
```

Glu Ala Val Ser Arg Val Leu Asn Lys Leu Gly Gly Val Glu Phe Asn
20 25 30

Ile Asp Leu Pro Asn Lys Lys Val Cys Ile Glu Ser Glu His Ser Ser
35 40 45

Asp Ile Leu Leu Ala Thr Leu Asn Lys Thr Gly Lys Ala Val Ser Tyr
50 55 60

Leu Gly Pro
65

Claims

1. An isolated polynucleotide comprising a sequence selected from the group consisting of:
 - (a) a sequence recited in SEQ ID NO:1;
 - (b) a sequence recited in SEQ ID NO:3;
 - (c) nucleotide sequences that hybridize to a polynucleotide complementary to a sequence recited in SEQ ID NO:1 and/or SEQ ID NO:3 under moderately stringent conditions, wherein said nucleotide sequence encodes a polypeptide having an ability to enhance cell survival; and
 - (c) nucleotide sequences that encode a polypeptide encoded by any of the foregoing sequences.
2. A recombinant expression vector comprising a polynucleotide according to claim 1.
3. A host cell transformed or transfected with an expression vector according to claim 2.
4. An isolated polynucleotide encoding a polypeptide recited in SEQ ID NO:2 or SEQ ID NO:4, or a variant of said polypeptide that is capable of enhancing cell survival.
5. A recombinant expression vector comprising a polynucleotide according to claim 4.
6. A host cell transformed or transfected with an expression vector according to claim 5.
7. A method for preparing an A6LA5A12 polypeptide, the method comprising culturing a host cell according to claim 3 or claim 6 under conditions promoting expression of the polynucleotide sequence and recovering an A6LA5A12 polypeptide.
8. A polypeptide comprising an amino acid sequence encoded by a polynucleotide according to claim 1.

9. A polypeptide comprising an amino acid sequence encoded by a polynucleotide according to claim 4.

10. A polypeptide comprising an amino acid sequence recited in SEQ ID NO:2 or SEQ ID NO:4, or a variant of said sequence, wherein the polypeptide is capable of enhancing cell survival.

11. A method for enhancing survival of a cell, the method comprising increasing A6LA5A12 activity within a cell, and thereby enhancing survival of the cell.

12. A method according to claim 11, wherein A6LA5A12 activity is increased by inducing expression of an endogenous A6LA5A12 gene.

13. A method according to claim 11, wherein A6LA5A12 activity is increased by administering a modulating agent.

14. A method according to claim 12, wherein the endogenous A6LA5A12 gene is induced by providing an activating agent to the cell.

15. A method according to claim 14, wherein the activating agent is a cytokine.

16. A method according to claim 14, wherein the activating agent is beta amyloid.

17. A method according to claim 12, wherein the endogenous A6LA5A12 gene is induced by providing an activating agent to the cell.

18. A method for enhancing survival of a cell, the method comprising increasing, within a cell, the level of a polypeptide according to claim 8 or claim 9.

19. A method for enhancing survival of a cell, the method comprising:

(a) transfecting the cell with a polynucleotide according to claim 1 or claim 4; and

(b) promoting expression of the polynucleotide within the cell, thereby enhancing survival of the cell.

20. A pharmaceutical composition comprising a polypeptide according to any of claims 8-10 in combination with a pharmaceutically acceptable carrier.

21. A pharmaceutical composition according to claim 20, for use in the manufacture of a medicament for treating a patient afflicted with a condition associated with oxidative damage.

22. A composition according to claim 21, wherein the condition is selected from the group consisting of CNS disorders, autoimmune diseases, arthritis, respiratory diseases, inflammatory conditions, skin disorders, cancer and viral infections.

23. A pharmaceutical composition according to claim 20, for use in the manufacture of a medicament for treating a patient afflicted with a condition associated with cell death induced by stress.

24. A composition according to claim 23, wherein the condition is selected from the group consisting of CNS disorders, autoimmune diseases, arthritis, respiratory diseases, inflammatory conditions, skin disorders, cancer and viral infections.

25. A composition according to claim 21 or claim 23, wherein the condition is associated with a defect in superoxide dismutase activity.

26. A method for identifying an agent that modulates A6LA5A12 activity, comprising:

(a) contacting a candidate modulator with a polypeptide according to claim 1 or claim 4, wherein the step of contacting is carried out under conditions and for a time sufficient to allow the candidate modulator to interact with the polypeptide; and

(b) subsequently identifying a modulator that binds to said polypeptide, and therefrom identifying an agent that modulates A6LA5A12 activity.

27. A method for modulating A6LA5A12 activity in a cell, comprising contacting a cell expressing a polypeptide according to claim 1 or claim 4 with an effective amount of an agent that modulates A6LA5A12 activity.

28. An antibody that binds to a polypeptide according to claim 1 or claim 4.

CACCGCGNCN ACACCGCCGC TGCCTCAGTC ATGCCGAAGC ACGAGTTCTC TGTGGACATG	60
ACCTGTGGAG GCTGTGCTGA AGCTGTCTCT CGGGTCCTCA ATAAGCTTGG AGGAGTTAAG	120
TATGACATTG ACCTGCCCAA CAAGAAGGTC TGCATTGAAT CTGAGCACAG CATGGACACT	180
CTGCTTGCAA CCCTGAAGAA AACAGGAAAG ACTGTTTCCT ACCTTGGCCT TGAGTAGCAG	240
GGGCCTGGTC CCCACAGCCC ACAGGATGGA CCAAAGGGGG CAGGATGCTG ATCCTCCCGC	300
TGGCTTCCAG ACAGACCTGG GACTTGGCAG TCATGCCGGG TNGATGGTGT TCCTGCGGAG	360
ACCCTCAAGT TTGTCCTATT CCTTTCCTAG CTTCCCTGCA ATAAAATCAA GCTGCTTTTG	420
TTGGA	425

FIG. 1

Met Pro Lys His Glu Phe Ser Val Asp Met Thr Cys Gly Gly Cys Ala
1 5 10 15
Glu Ala Val Ser Arg Val Leu Asn Lys Leu Gly Gly Val Lys Tyr Asp
20 25 30
Ile Asp Leu Pro Asn Lys Lys Val Cys Ile Glu Ser Glu His Ser Met
35 40 45
Asp Thr Leu Leu Ala Thr Leu Lys Lys Thr Gly Lys Thr Val Ser Tyr
50 55 60
Leu Gly Leu Glu
65

FIG. 2

CTGTGCCGCC TGTGCCGCCT GTGCCGCGTC AGTCATGCCG AAGCACGAGT TCTCTGTGGA	60
CATGACCTGT GGAGGCTGTG CGGAAGCCGT CTCCCGAGTC CTCAACAAGC TGGGAGGAGT	120
GGAGTTCAAC ATTGACTTGC CCAACAAGAA GGTCTGCATC GAGTCTGAGC ACAGCTCAGA	180
CATCCTGCTG GCAACTCTCA ACAAACAGG AAAAGCGGTC TCCTACCTTG GCCCCAAGTA	240
GCCAGGACCT GGGCGAGTCC TTCCGGATAG AAAGTGAAGA GGCAGACTGT TGATCTGGTC	300
TCCCCCGCTG ATCTGGAACA CCAACTGCTC AGTCCAGCTC CGCCATGGAA TTCCAGCCCA	360
GACAGGCCTT CCCTGCTGGC TCCCTGCCAG CTTCTTGCA ATTAAAGTCA AGCTGCATTT	420
GTAAAAAAA AAAAAAAAAA AAAA	444

FIG. 3

Met Pro Lys His Glu Phe Ser Val Asp Met Thr Cys Gly Gly Cys Ala
1 5 10 15
Glu Ala Val Ser Arg Val Leu Asn Lys Leu Gly Gly Val Glu Phe Asn
20 25 30
Ile Asp Leu Pro Asn Lys Lys Val Cys Ile Glu Ser Glu His Ser Ser
35 40 45
Asp Ile Leu Leu Ala Thr Leu Asn Lys Thr Gly Lys Ala Val Ser Tyr
50 55 60
Leu Gly Pro
65

FIG. 4

$\Delta 6$ LA5A12 Acts as a Survival Factor for Serum Starved Neurons

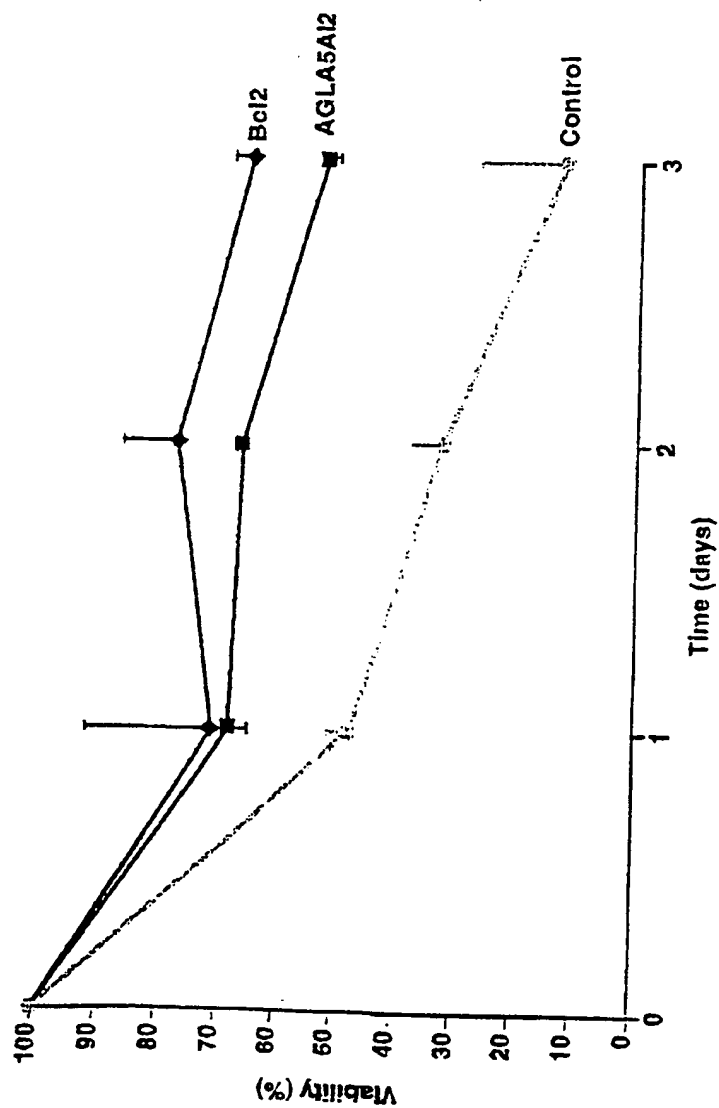


FIG. 5

**AGLA5A12 mRNA Expression in
Ischemia (4hr post-MCAO)**

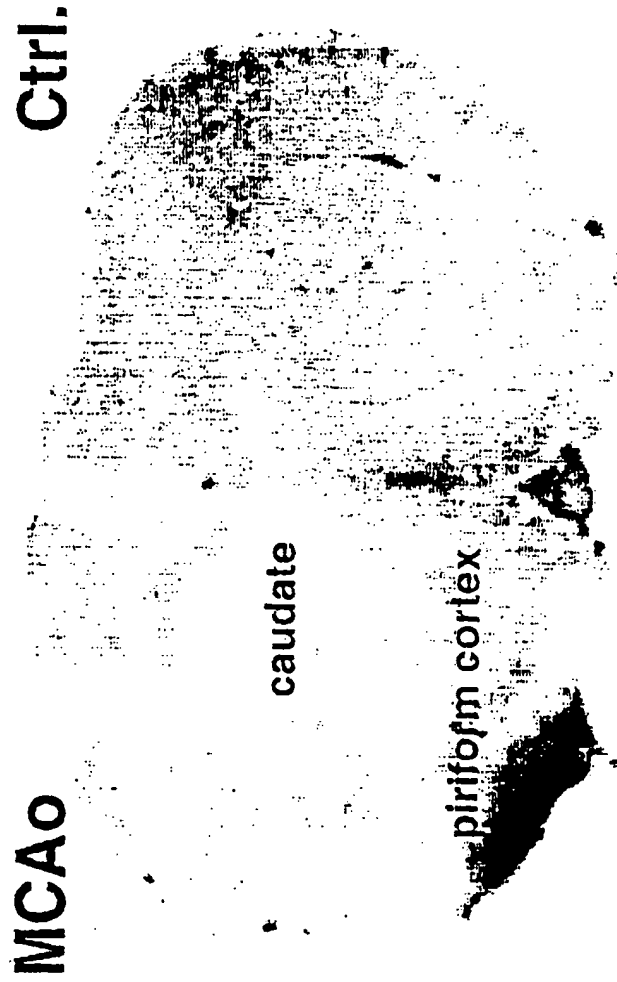


FIG. 6

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